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Research Note

Salmonella Prevalence in Bovine Lymph Nodes Differs among Feedyards

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ABSTRACT

Lymphatic tissue, specifically lymph nodes, is commonly incorporated into ground beef products as a component of lean trimmings. *Salmonella* and other pathogenic bacteria have been identified in bovine lymph nodes, which may impact compliance with the *Salmonella* performance standards for ground beef established by the U.S. Department of Agriculture. Although *Salmonella* prevalence has been examined among lymph nodes between animals, no data are currently available regarding feedyard origin of the cattle and *Salmonella* prevalence. Bovine lymph nodes (279 superficial cervical plus 28 iliofemoral = 307) were collected from beef carcasses at a commercial beef harvest and processing plant over a 3-month period and examined for the prevalence of *Salmonella*. Cattle processed were from seven feedyards (A through G). *Salmonella* prevalence was exceptionally low (0% of samples were positive) in cattle from feedyard A and high (88.2%) in cattle from feedyard B. Prevalence in the remaining feedyards ranged widely: 40.0% in feedyard C, 4.0% in feedyard D, 24.0% in feedyard E, 42.9% in feedyard F, and 40.0% in feedyard G. These data indicate the range of differences in *Salmonella* prevalence among feedyards. Such information may be useful for developing interventions to reduce or eliminate *Salmonella* from bovine lymph nodes, which would assist in the reduction of *Salmonella* in ground beef.

Lymph nodes are commonly found in lean trimmings destined for ground beef production. Lymphatic tissue, specifically lymph nodes, has been identified as a potential source of pathogenic bacteria *(2)*. Most previous studies have been focused on *Salmonella* in mesenteric lymph nodes *(4, 5)*. However, in some studies the prevalence of *Salmonella (2)* and other bacteria *(3)* has been analyzed in lymph nodes destined for use in ground product as a component of lean trimmings. Although contradicting bacterial prevalence data have been reported, research has been focused on prevalence among types of lymph nodes rather than on the origin or source of the cattle. In the most recent research *(2), Salmonella* prevalence in lymph nodes potentially destined for ground products was low.

The present study evolved from an effort to identify the possible cause of periodic increases in *Salmonella* prevalence in a commercial beef harvest and processing establishment. After multiple years of collecting data, including carcass mapping, environmental factors, weather patterns, and other processing data, the management of this establishment speculated that the feedyard source of cattle might be related to *Salmonella* prevalence. After monitoring *Salmonella* data over time and focusing on how these data related to cattle origin, the potential for variation in *Salmonella* presence among feedyards was suggested. With limited data available in this field of research, the present study was designed to determine whether *Salmonella* prevalence in bovine lymph nodes differed among cattle from different feedyards.

MATERIALS AND METHODS

Sample collection. Three hundred seven bovine lymph nodes were obtained from beef carcasses at a commercial beef harvest and processing establishment. Four collection trips were conducted over a 3-month period (July through September). Each collection trip was designed to obtain lymph nodes from preselected feedyards in the southern United States. The superficial cervical ($n = 279$) and iliofemoral ($n = 28$) lymph nodes were analyzed for this study. Superficial cervical lymph nodes were excised from unchilled carcasses that had been transferred from the harvest floor to the blast-chill cooler. Approximately one-half of the superficial cervical lymph nodes were excised from left sides and the other half were excised from right sides of the carcasses. Iliofemoral lymph nodes were collected from chilled carcasses during fabrication (approximately 24 to 48 h postmortem). Following excision, fat-encased lymph nodes were placed in labeled Whirl-pak bags (Nasco, Modesto, CA) and transported for processing to the Texas A&M University Food Microbiology Laboratory (College Station) in an insulated container with refrigerant packs. Upon arrival in the laboratory, lymph nodes were removed from the insulated container and stored under refrigeration (4°C) until processing.

Sample processing. All lymph nodes ($n = 307$) were aseptically trimmed free of fat and flame sterilized within 24 h of

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collection. Aseptic technique was maintained by first immersing the entire fat-encased lymph node in 95% ethanol and then flame sterilizing the outside surface before removing the fat. A scalpel and forceps were flame sterilized before and after each cut used to remove the fat. After fat removal, the fully exposed lymph nodes were flame sterilized. For the first set of lymph nodes collected ($n = 57$), a flame-sterilized scalpel and forceps were used to aseptically pulverize samples by mincing each lymph node to expose the interior node material. Because of laboratory constraints, lymph nodes from the first collection trip were the only samples analyzed at the Center for Food Safety (Texas A&M University Food Microbiology Laboratory).

All other samples were processed as described above to aseptically extract the fat-encased lymph node and then individually packaged in Whirl-pak bags and transported in an insulated container with refrigerant packs to the U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Food and Feed Safety Research Center (College Station, TX) for pulverization and analysis. Upon receipt of samples at USDA-ARS Center, each lymph node was transferred aseptically to a filtered Whirl-pak bag and pulverized with a rubber mallet.

Prevalence determination. Lymph nodes excised on the first collection trip were analyzed using a fully automated VIDAS system (bioMérieux, Hazelwood, MO) *(1)*. This system utilizes the enzyme-linked fluorescent assay method based on the specific phage capture technology and replaces traditional enrichment methods. Both motile and nonmotile *Salmonella* cells can be detected. Each minced lymph node was placed in a sterile stomacher bag with 225 ml of buffered peptone water (Difco, BD, Sparks, MD) and 1 ml of *Salmonella* phage technology (SPT) supplement containing brilliant green and novobiocin (bioMérieux). Samples were pummeled for 1 min with a Stomacher 400 (Tekmar Company, Cincinnati, OH) and then incubated at 41.5°C for 22 to 26 h. A 0.5-ml aliquot of each enriched sample was introduced to a VIDAS SPT test strip containing predispensed reagents. Inoculated test strips were heated for 5 min at 100°C using a VIDAS Heat and Go unit and then allowed to cool for 10 min. Prepared test trips then were placed into a VIDAS automated immunoanalyzer for analysis within 48 min. Positive samples were those with a test value ≥ 0.25. Presumptive *Salmonella*-positive cultures were confirmed by isolation on ChromID *Salmonella* (bioMérieux) after incubation for 24 h at 37°C. Light pink to mauve colonies were confirmed as *Salmonella* according to a USDA Food Safety and Inspection Service method *(6)* by streaking onto triple sugar iron agar and lysine iron agar (Difco, BD) slants. Tubes were incubated at 35°C for 24 h, and those isolates typical of *Salmonella* were further confirmed by a combination of biochemical and serological procedures. For serological confirmation, isolates were tested with polyvalent O antiserum reactive with serogroups A through I and Vi (Difco, BD). Those isolates that were positive for agglutination also were confirmed biochemically using commercially available API 20E (bioMérieux) kits following the manufacturer's instructions. According to the VIDAS UP *Salmonella* (SPT) package insert, the relative detection level for the 50% detection limit is between 0.3 and 1.3 cells per 25 g and the sensitivity is 96.8%.

Lymph nodes analyzed at the USDA-ARS Center were prepared by adding 100 ml of tetrathionate broth (Difco, BD) to each filtered Whirl-pak bag and hand massaging the mixture for approximately 1 min. After incubation for 24 h at 37°C, 100 μl of the enriched culture was transferred to 5 ml of Rappaport-Vassiliadis broth (Difco, BD) and incubated an additional 24 h at 42°C. Ten microliters of this enriched culture was streaked onto brilliant green agar (Difco, BD) containing 25 μg/ml novobiocin and incubated for 24 h at 37°C. Suspect colonies were picked and transferred to triple sugar iron slants (Difco, BD), and presumptive-positive slants were further confirmed as *Salmonella* using slide agglutination with *Salmonella* antiserum (Difco, BD). The limit of detection was 10^2 CFU/g of tissue.

RESULTS AND DISCUSSION

The first collection trip was organized to obtain a total of 57 bovine lymph nodes (29 superficial cervical and 28 iliofemoral) from cattle from four different feedyards. The intent was to collect and analyze 60 lymph nodes; however, three lymph nodes were compromised and excluded from analysis. More samples were collected from the primary feedyard of concern (feedyard F; 14 superficial cervical and 14 iliofemoral) than from the other three feedyards, which were chosen at random (feedyard A, 5 superficial cervical and 4 iliofemoral; feedyards B and G, 5 superficial cervical and 5 iliofemoral). After reviewing the results from the first collection, two interesting findings were noted. Of the four feedyards sampled, feedyard A provided no *Salmonella*-positive samples from both the superficial cervical and iliofemoral lymph nodes. In contrast, for feedyard B 100.0% of samples from superficial cervical lymph nodes and 80% of samples from iliofemoral lymph nodes were positive for *Salmonella,* for a cumulative 88.2% positive lymph nodes (Table 1). From the feedyard initially identified by the processing establishment as the primary source of concern (feedyard F), 42.9% of the samples were positive for *Salmonella* (Table 1), and no lymph nodes were collected from cattle from this feedyard on subsequent collection trips.

A second trip was made 50 days later to collect 25 superficial cervical lymph nodes from each of feedyards A and B and from two additional feedyards (C and D). Results from the second trip were again 0% *Salmonella*-positive samples for feedyard A and 100.0% *Salmonella*-positive samples for feedyard B; 40.0 and 8.0% of samples from cattle of feedyards C and D, respectively, were positive for *Salmonella*. These results corroborate those of the first collection trip, providing evidence that cattle from the two feedyards clearly differed with regard to *Salmonella* prevalence; however, the reason for these differences remains unknown.

A third trip was made 15 days after the second trip to determine whether the apparent difference in prevalence among yards remained. A total of 100 lymph nodes were collected from feedyards A, B, D, and an additional feedyard (E). No lymph nodes from feedyards A and D were positive for Salmonella, whereas 76.0 and 24.0% of lymph nodes from feedyards B and E, respectively, were positive for *Salmonella*. With the clear distinction between the same two feedyards being repeated, we began to inquire as to what contribution, if any, the country of origin of the cattle may make to these differences in *Salmonella* prevalence.

To address differences in prevalence due to country of origin, a fourth and final collection trip was made 4 days after the third trip, and 25 lymph nodes were collected from each of feedyards A and B. This collection focused on cattle solely of Mexican origin, whereas all other collections were made from cattle of U.S. origin. Similar results were found

TABLE 1. *Percentage of* Salmonella*-positive lymph nodes by feedyard for each collection trip and cumulatively*

	% (no. postive/no. tested) *Salmonella*-positive lymph nodes				
Feedyard	Collection 1	Collection 2	Collection 3	Collection 4	Total
A	0.0 (0/9)	0.0 (0/25)	0.0 (0/25)	0.0 (0/25)	0.0 (0/84)
B	90.0 (9/10)	100.0 (25/25)	76.0 (19/25)	88.0 (22/25)	88.2 (75/85)
C	NC[a]	40.0 (10/25)	NC	NC	40.0 (10/25)
D	NC	8.0 (2/25)	0.0 (0/25)	NC	4.0 (2/50)
E	NC	NC	24.0 (6/25)	NC	24.0 (6/25)
F	42.9 (12/28)	NC	NC	NC	42.9 (12/28)
G	40.0 (4/10)	NC	NC	NC	40.0 (4/10)

[a] NC, no lymph nodes were collected from these feedyards on these collection trips.

(0.0% positive samples from feedyard A; 88.0% positive samples from feedyard B), further indicating the potential influence of feedyard on *Salmonella* prevalence in lymph nodes.

Cumulative percentages of *Salmonella*-positive lymph nodes across collections and feedyards are shown in Table 1. The prevalence of *Salmonella* among feedyards was markedly different, especially between feedyards A and B. The present study provides the basis for additional research. Specific items for consideration may include cattle type and temperament, cattle stress levels and exposure, veterinary treatments administered, and preharvest interventions employed. Of greatest importance will be the investigation of practices and environmental factors that may be contributing to the complete absence of *Salmonella* in the lymph nodes of cattle from feedyard A versus continued presence of this pathogen in cattle from other feedyards.

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